イノベーションに情熱を。
ひとに思いやりを。

Daiichi-Sankyo

第一三共グループにおける研究開発生産性向上の取組み

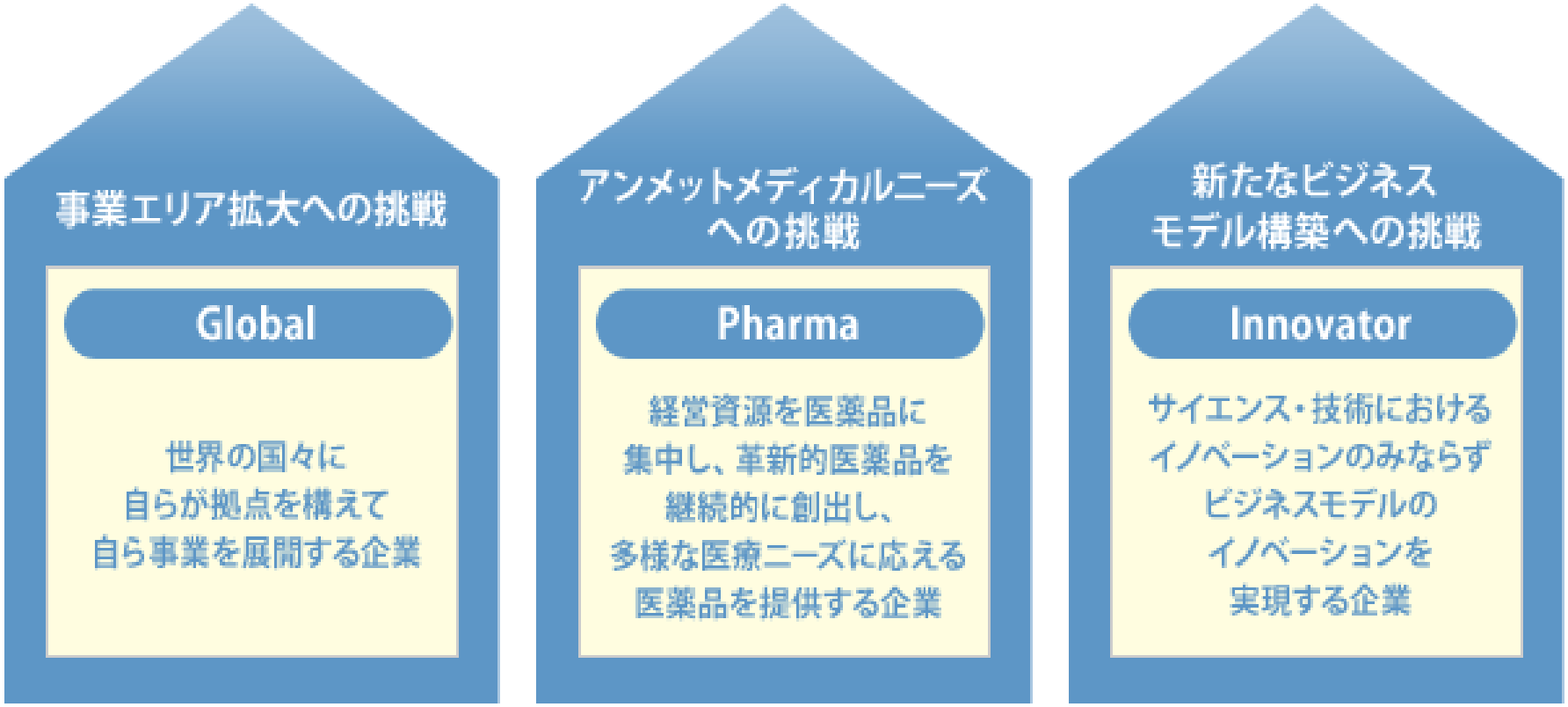
2012年10月24日

第３回JSPS研究開発専門委員会

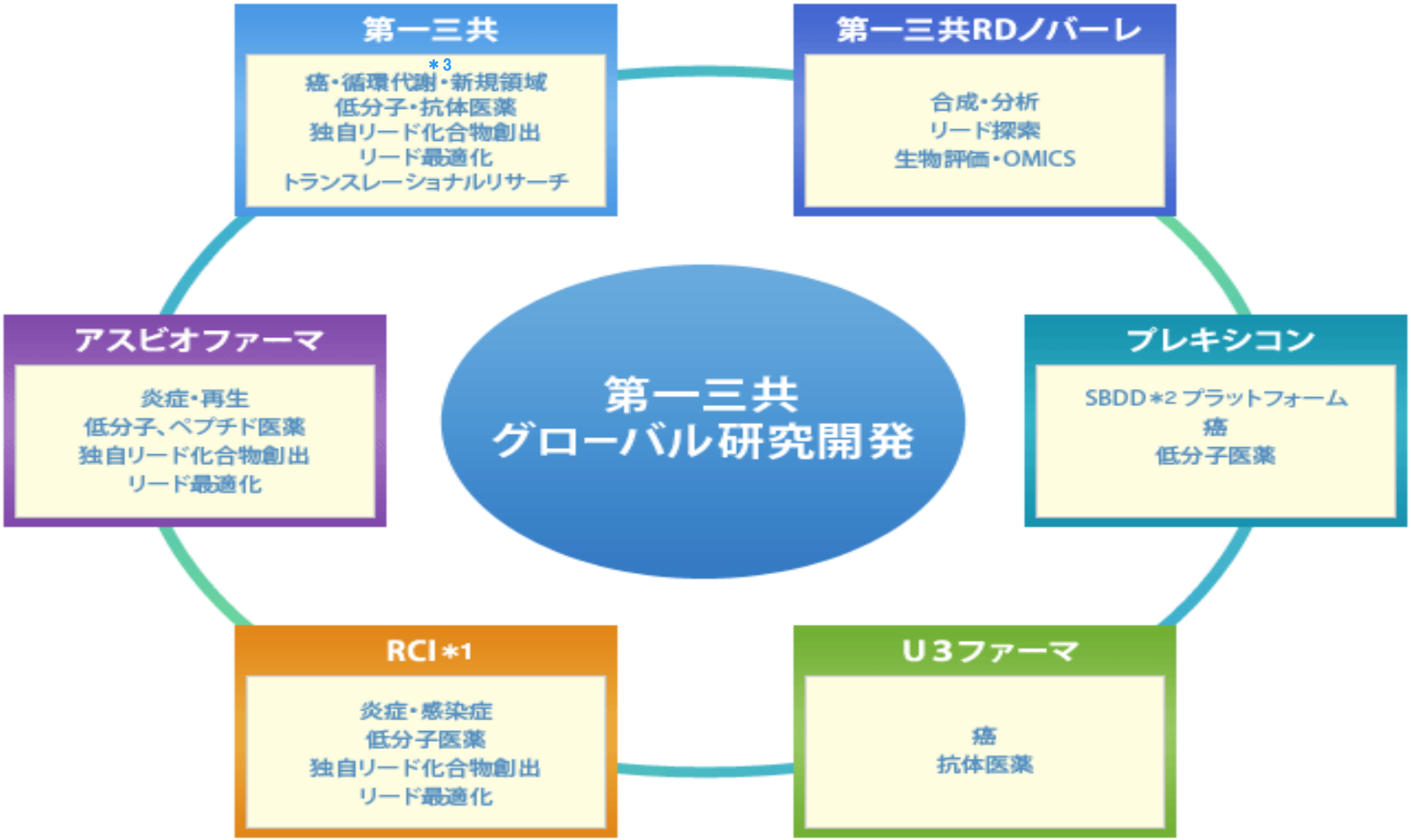
春山英幸
第一三共RDノバーレ株式会社

第一三共の2015年ビジョン

2015年に第一三共グループが目指す企業像
Global Pharma Innovator の実現



グローバル研究機能



＊1 RCI ： Daiichi Sankyo Life Science Research Centre in India
＊2 SBDD: Scaffold-Based Drug discovery
＊3 循環代謝: 心臓・血管系疾患に関わる代謝性疾患

重点領域の絞り込み

Key Message　未充足医療ニーズの高い重点領域での競争力の向上

*FIC: First in Class

RD ノバーレは、第一三共グローバルRDの共通創薬基盤プラットフォームとして発足

- プロセスを集約することで習熟効果が働き、効率・生産性が上がる
- 分離することのデメリットに比してメリットが大きい
- プログラム/プロジェクト固有の技術で無い
- 活動のアウトプットが、プロダクトのIPそのものではない

2011年4月

研究開発本部

- **疾患領域に特化した機**
  癌、循環代謝、先端医

- **共通基盤技術機能**;
ADME, Tox/ HTS/ 蛋白発現/
天然物/オミックス技術

RD アソシエ;
研究開発支援機能
化学合成/分析業務/生物評価/
臨床開発（モニター業務）

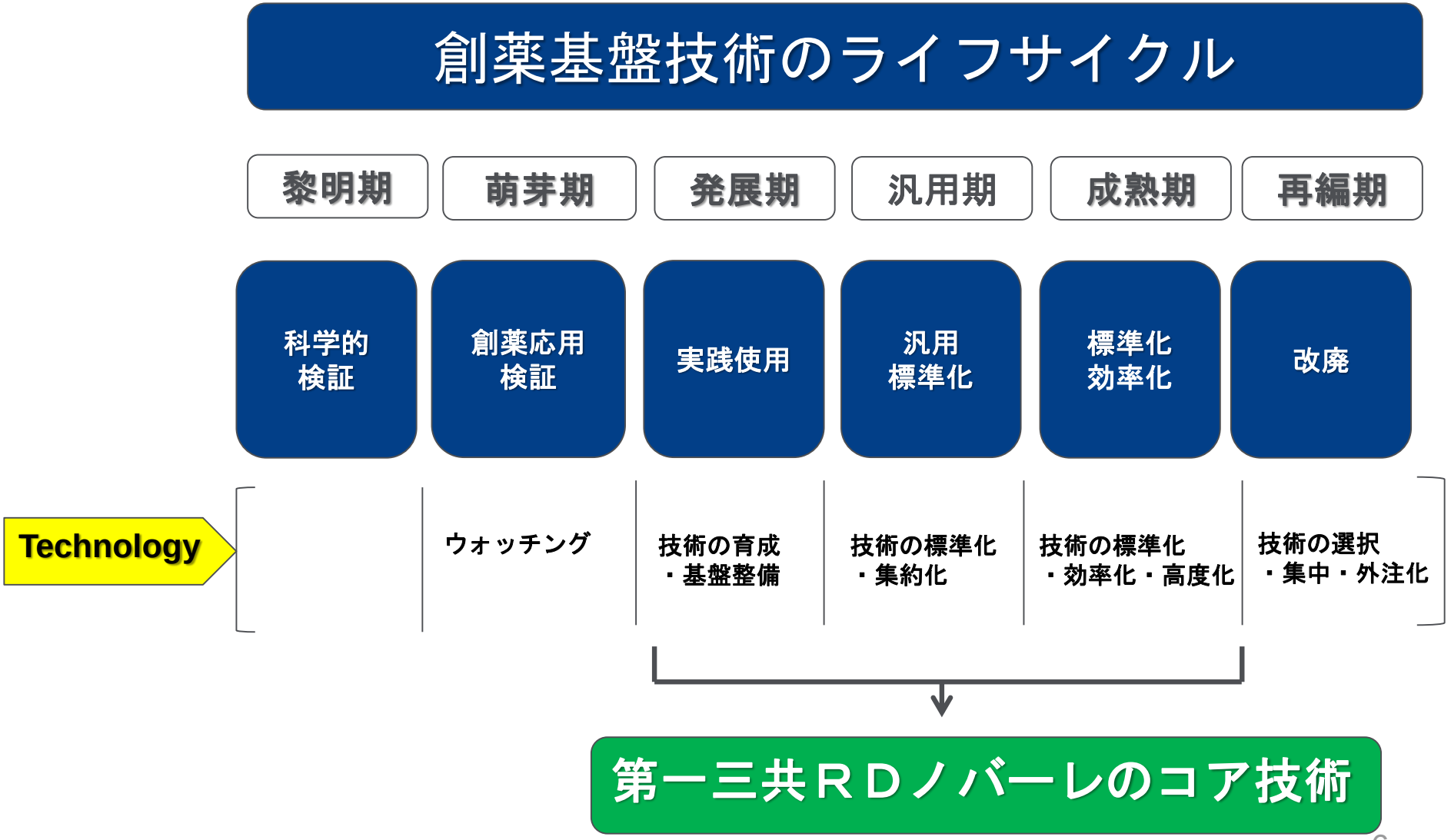
2011年10月

開発本部
患領域に特化した機能;
循環代謝、先端医薬

共通基盤技術機能;
前臨床研究

RD ノバーレ;
FIC創薬のためのソリューションプロバイダー
- 初期探索研究のための統合技術プラットフォーム
- 臨床開発運営プラットフォーム（POC取得以降）

第一三共RDノバーレのコア技術



ヒト組織中の薬物代謝酵素同定のための proteomic correlation profilingの応用

Proteomic correlation profiling to identify a drug-metabolizing enzyme in human tissue

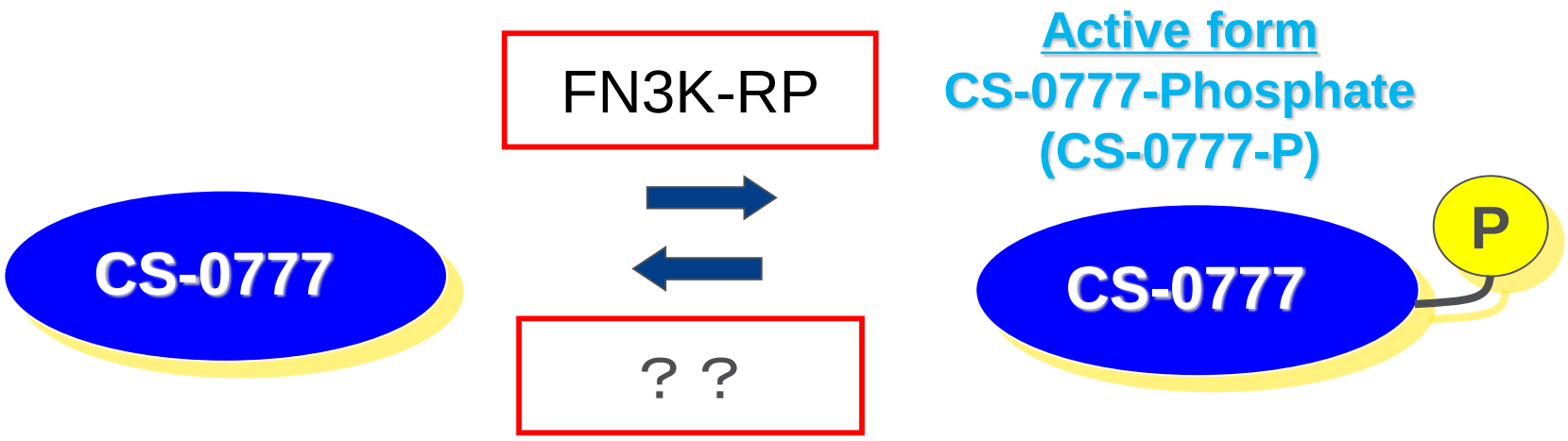
Hedetaka Sakurai[1], Kazuishi Kubota[1], Shin-ichi Inaba[2], Kaoru Takanaka[2], Akira Shinagawa[1]

[1]DAIICHI SANKYO RD NOVARE CO., LTD, [2]DAIICHI SANKYO CO., LTD

日本プロテオーム学会2012年年会
2012年7月26、27日

Introduction

- CS-0777 and Fingolimod need phosphorylation to have a $S1P_1$ agonist activity.
- Fingolimod was approved as a first oral drug for multiple sclerosis by FDA in September 2010.
- The kinase and the phosphatase for Fingolimod have been already reported.
- The kinase for CS-0777 identified by our team is different from a Fingolimod kinase.
- **The phosphatase for CS-0777 has not been identified so far**.
- Identification of the phosphatase is required to fully understand mechanism of activation and inactivation of CS-0777.



Purification - 1

Human kidney 1.1 g homogenate

100,000 *g*

pellet

sup (#1)

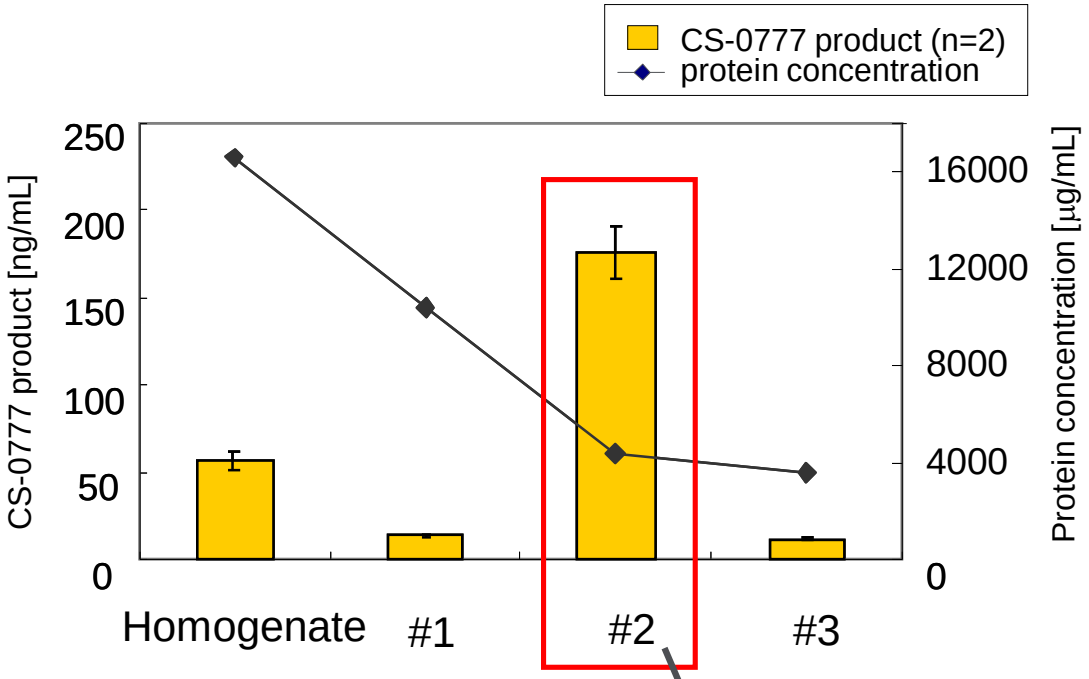
Solubilized with 1% DM

15,000 *g*

sup (#2)

pellet (#3)

Fractionated human kidney homogenate



Used for purification

- CS-0777-P phosphatase activity mainly resided in the insolbule fraction.
- A detergent (1% DM) was found to be very effective in solubilizing the activity.

Purification - 2

#2 fraction obtained from Purification - 1

↓

Step 2
Multimodal anion-exchange
Adhere pH9

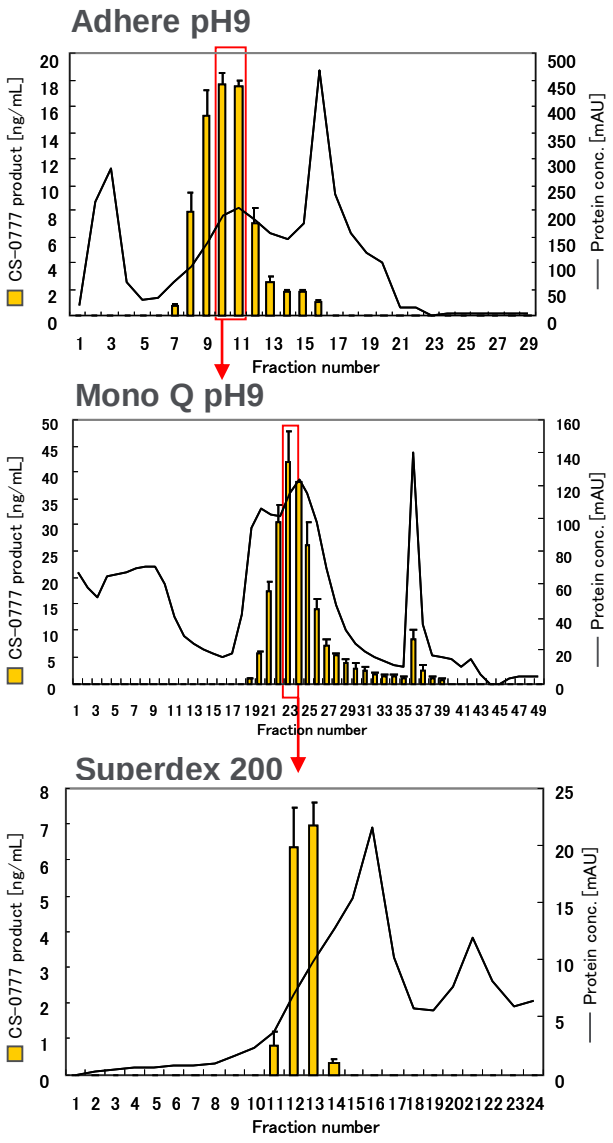
↓

Step 3
Anion-exchange
Mono Q pH9

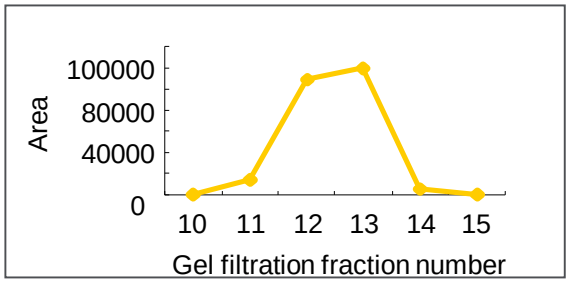
↓

Step 4
Gel Filtration
Superdex 200

Enzyme activity was concentrated by about 200-fold.

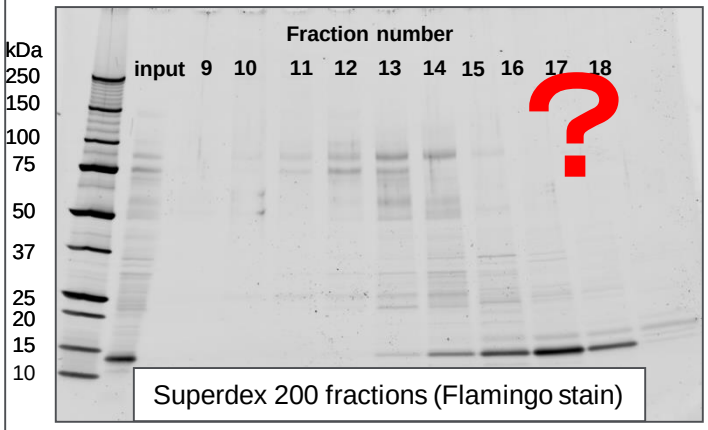


Phosphatase activity



We were not able to find the band that correlated with CS-0777-P phosphatase activity by this purification.

Gel stain



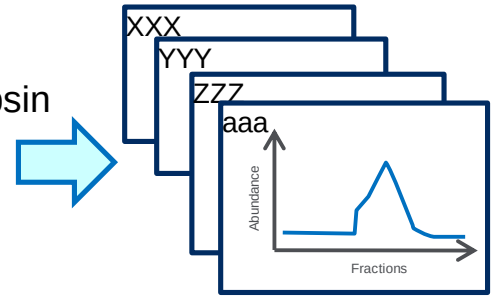
Identification of candidate proteins by Proteomic Correlation Profiling*

Gel filtration of the enzyme active fractions

Proteins digested by trypsin

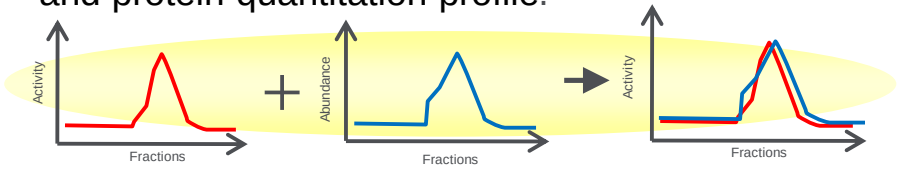
LC-MS/MS

Database search



Identified 274 proteins and MS-based protein quantitation

Draw profile comparisons phosphatase activity profile and protein quantitation profile.



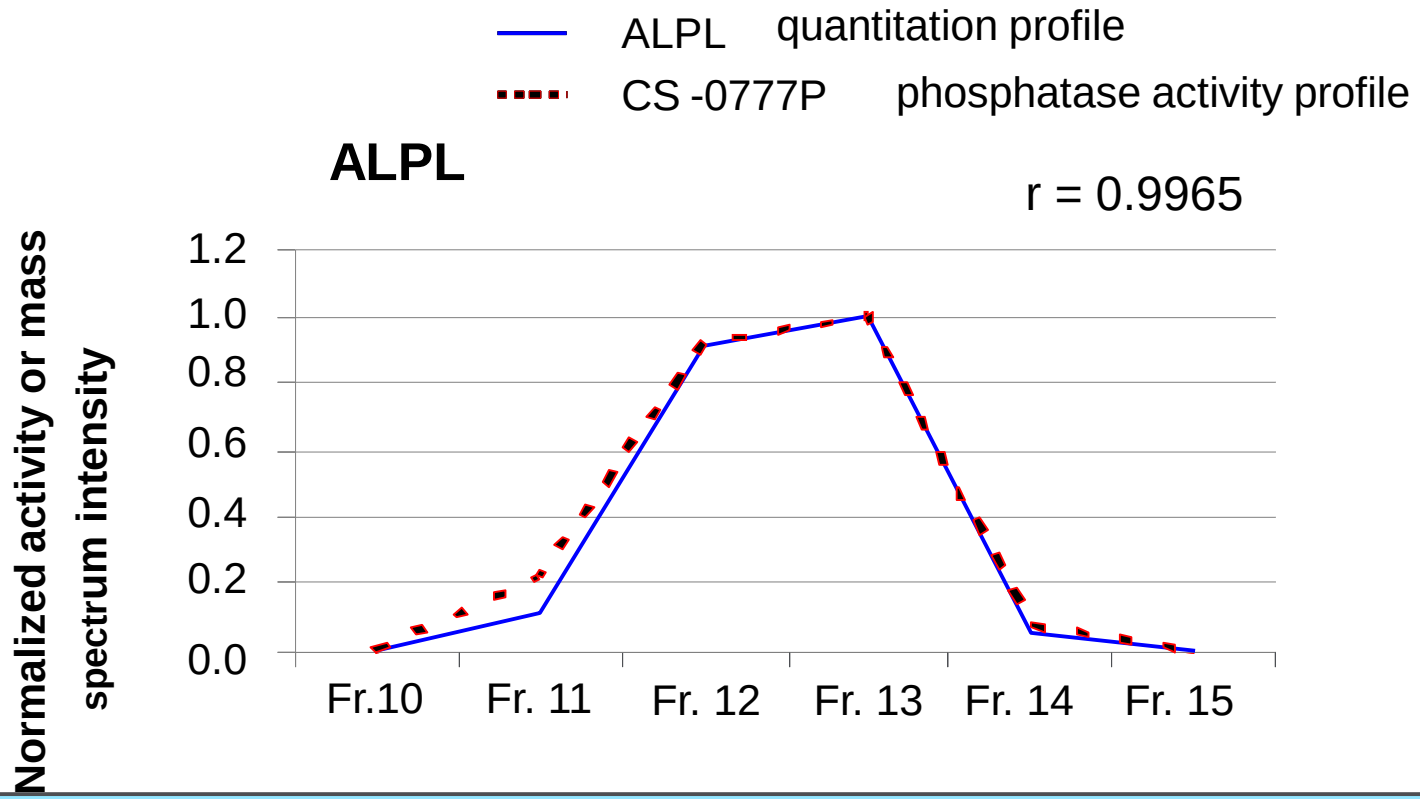
Rank	Gene symbol	Correlation coefficient (*r*)
1	SLC13A2	0.9984
3	SLC2A1	0.9958
4	NCSTN	
5	SLC22A6	
6	SLC22A8	0.9835
7	SLC17A3	0.9817
8	SLC22A12	0.9742
9	HADHA	0.9741
10	FH	0.9739
11	BSG	0.9682
12	LAMP1	0.9277
13	HADHB	0.9226
14	SLC5A12	0.9182
15	XPNPEP2	0.8871
16	DPP4	0.8855
17	C9orf46	0.8673
18	MME	0.8513
19	ATP1B3	0.7816
19	SLC22A12	0.7816

Rank 2 / 274

The Pearson correlation between the protein amount and CS-0777-P phosphatase activity for all 274proteins was calculated.

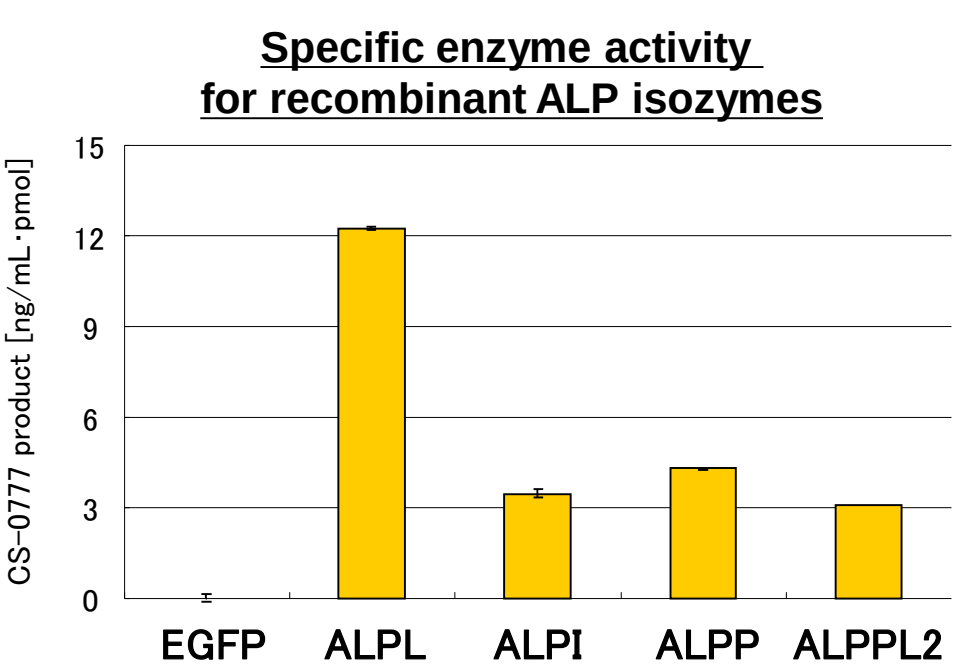
* Kubota K. et al. (2009) *Nat. Biotechnol.* **27**, 933-940

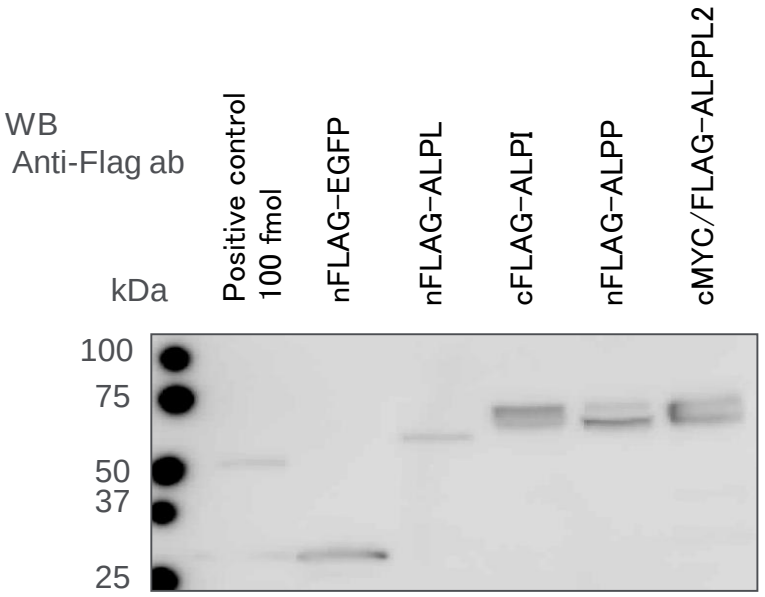
Identification of candidate proteins by Proteomic Correlation Profiling



Alkaline phosphatase activities for , tissue none specific isozyme (ALPL) showed best correlation between phosphatase activity and MS-based quantitation as phosphatases.

CS-0777-P phosphatase activity of recombinant ALP isozymes





Recombinant all ALP isozymes had CS-0777-P phosphatase activity *!*

In particular, ALPL had 3-fold higher specific activity compared to other ALP isozymes.
ALPL: Tissue-nonspecific isozyme (Liver/Bone/Kidney), **ALPI**: Intestinal isozyme, **ALPP**: Placental isozyme, **ALPPL**: Placental like isozyme (Germ cell)

Gene Targeting Discovery of Novel Nucleoside Antibiotics

Gordon Research Conference, Natural Products
July 22-27, 2012

Masanori FUNABASHI[1], Satoshi BABA[2], Toshio TAKATSU[3], Masaaki KIZUKA[1], Masahiro TANAKA[1], Steven G. Van Lanen[4], Koichi NONAKA[5]

[1] Natural Product Research Group, Discovery Science and Technology Department, Daiichi Sankyo RD Novare Co., Ltd., Japan
[2] Biologics Research Laboratories, R&D division, Daiichi Sankyo Co., Ltd., Japan
[3] Analytical Chemistry Research Group, Center for Pharmaceutical and Biomedical Analysis, Daiichi Sankyo RD Novare Co., Ltd., Japan
[4] Division of Pharmaceutical Sciences, College of Pharmacy, University of Kentucky, USA
[5] Biopharmaceutical Technology Research Laboratories, Pharmaceutical Technology Division, Daiichi Sankyo Co., Ltd., Japan

DAIICHI SANKYO RD NOVARE CO., LTD.

Motivation

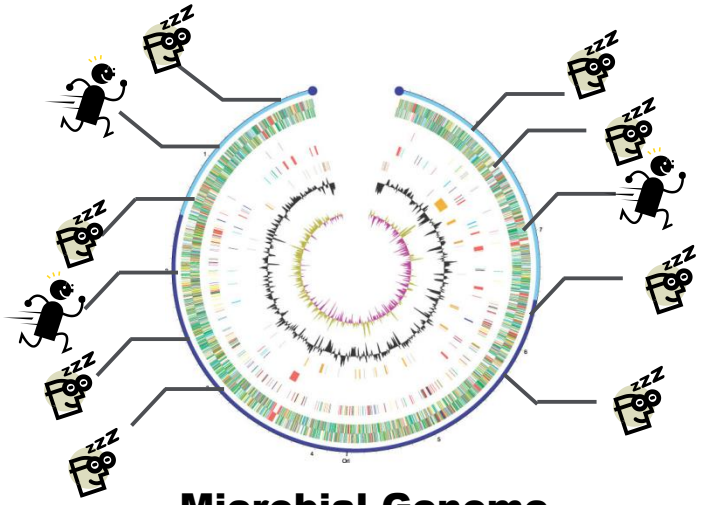
Context

The number of new drug leads from natural products has decreased

Is there a perception that the variety of natural product has run dry?

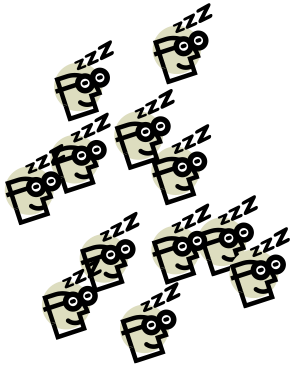
The Answer is No

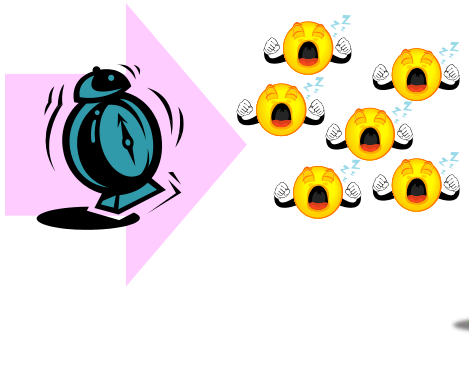
From recent microbial genomics research revealed that there is a huge number of 'silent' biosynthetic gene clusters



Microbial Genome

'Silent'
Biosynthetic Gene Clusters



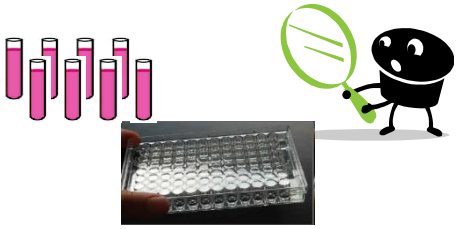


Treasure House of Drug Leads !

Approaches to discover new compounds

Daiichi-Sankyo

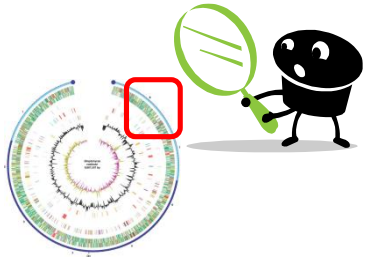
Traditional Strategy



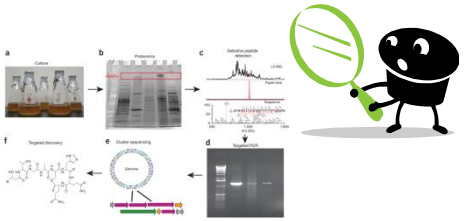
Bioassay-guided

Emerging Strategy

Genome Mining Approach

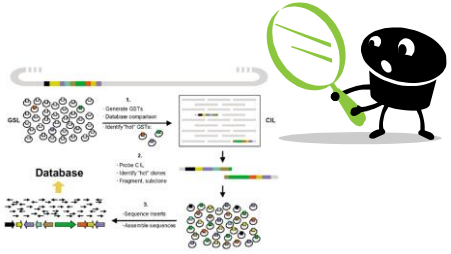


Proteomics Approach



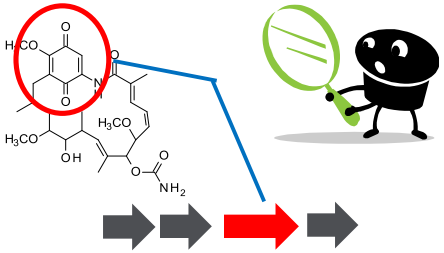
Bumpus S. B. *et. al.* Nat. Biotech. 27 951-956

Genome Scanning Approach



Zazopoulos E., *et. al.* Nat. Biotech. 21 187-190

Gene Targeting Approach



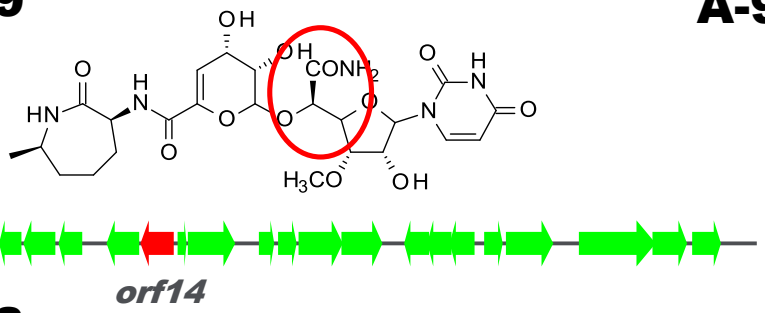
Motivation

Gene targeting approach for novel translocase I inhibitors

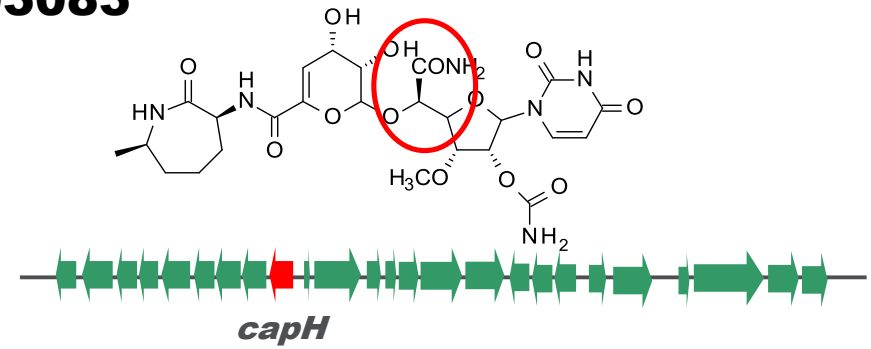
Hypothesis

We could discover strains which have a potential for producing translocase I inhibitor with uridine moiety by targeting the SHMT gene

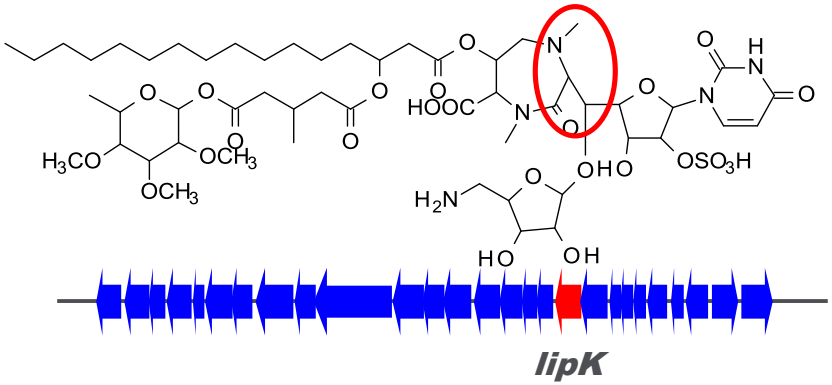
A-500359



A-503083



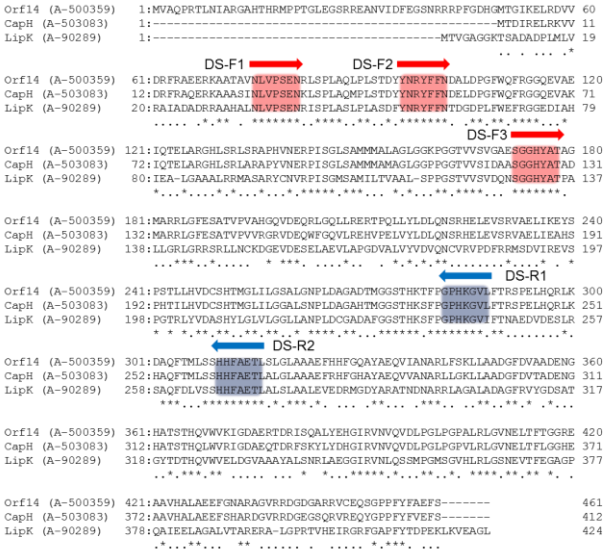
A-90289



Serine hydroxymethyltransferase (SHMT) homolog
is conserved in the uridine-nucleoside biosynthetic gene clusters (*orf14*, *capH* and *lipK*)
is a key enzyme for the biosynthesis of these uridine-nulceoside antibiotic

Results

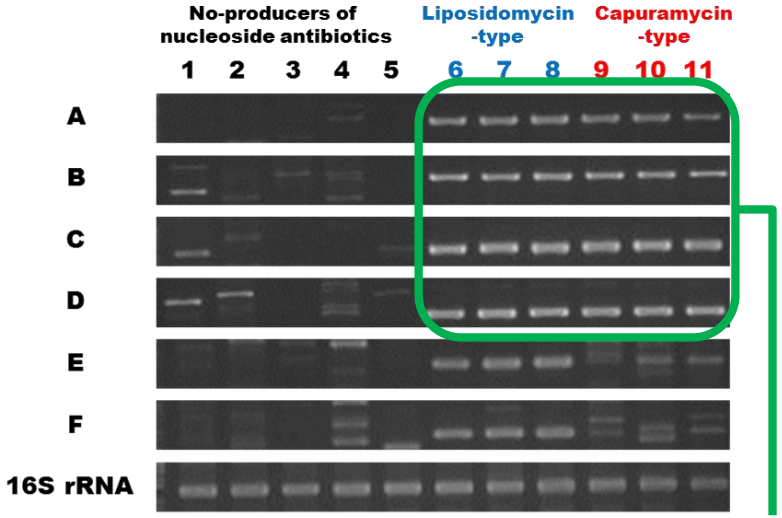
I. Construction of Degenerate Primers



Primer Sets
A is primer combination of DS-F1 and DS-R1,
B is of DS-F1 and DS-R2,
C is of DS-F2 and DS-R1,
D is of DS-F2 and DS-R2,
E is of DS-F3 and DS-R1,
F is of DS-F3 and DS-R2.

Set B was used for 1st screening
Set A and C were used for 2nd screening

II. Validation for PCR Condition



Specific for translocase I inhibitor producers

Lane: 1, *Streptomyces coelicolor* A3(2) ATCC BAA-471;
Lane: 2, *Streptomyces avermitillis* ATCC 31267;
Lane: 3, *Streptomyces gieseus* IFO 13350;
Lane: 4, *Saccharopolyspora erythraea* NRRL 3887;
Lane: 5, *Streptomyces lividans* TK21.
Lane: 6, *Streptomyces* sp. SANK 60405, A-90289 producer;
Lane: 7, *Streptomyces* sp. SANK 60704, A-97065 producer;
Lane: 8, *Streptomyces arenae* NRRL 2377, A-84830 producer.
Lane: 9, *Streptomyces griseus* SANK 60196, A-500359 producer;
Lane 10, *Streptomyces* sp. SANK 62799, A-503083 producer;
Lane 11, *Amycolatopsis* sp. SANK 60206, A-102395 producer.

Reaction Components

0.5 M	betaine
X1	Gotaq
0.4 μM	forward primer
0.4 μM	reverse primer
15%	crude genome extracted by InstaGene D.W.

Reaction Condition
35 cycles of [94˚C for 30 sec, 45˚C for 30 sec and 72˚C for 60 sec]

Results

III. PCR-based Screening

~2500 strains

1st → **rarely-explored actinomycetes isolated from soil**

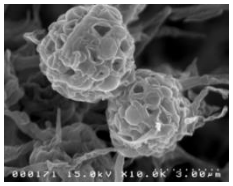
29

2nd

6

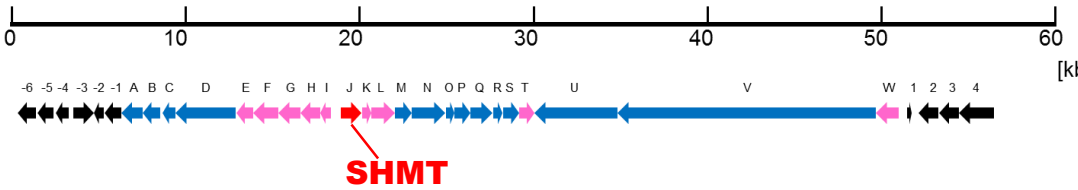
Sequence Check

1



***Sphaerisporangium* sp. SANK 60911**

IV. Sequence Analysis



SphA	Glycosyl transferase group 1	SphM	Sucraseferredoxin family protein
SphB	Arylsulfatase	SphN	Transketolase
SphC	Phosphoesterase	SphO	MmgE/PrpD family protein
SphD	ABC transporter	SphP	Aldo/keto reductase
SphE	Dioxygenase	SphQ	Diaminopimelate decarboxylase
SphF	Pyrimidine-nucleoside phosphorylase	SphR	Hypothetical Protein
SphG	Aminotransferase	SphS	F420-dependent oxidoreductase
SphH	Glycosyltransferase	SphT	Nucleotidyltransferase
SphI	Nucleotidyltransferase	SphU	Type I polyketide synthase
SphJ	Serine hydroxymethyltransferase	SphV	Type I polyketide synthase
SphK	β-hydroxylase	SphW	Non-ribosomal peptide synthethase
SphL	Aminotransferase		

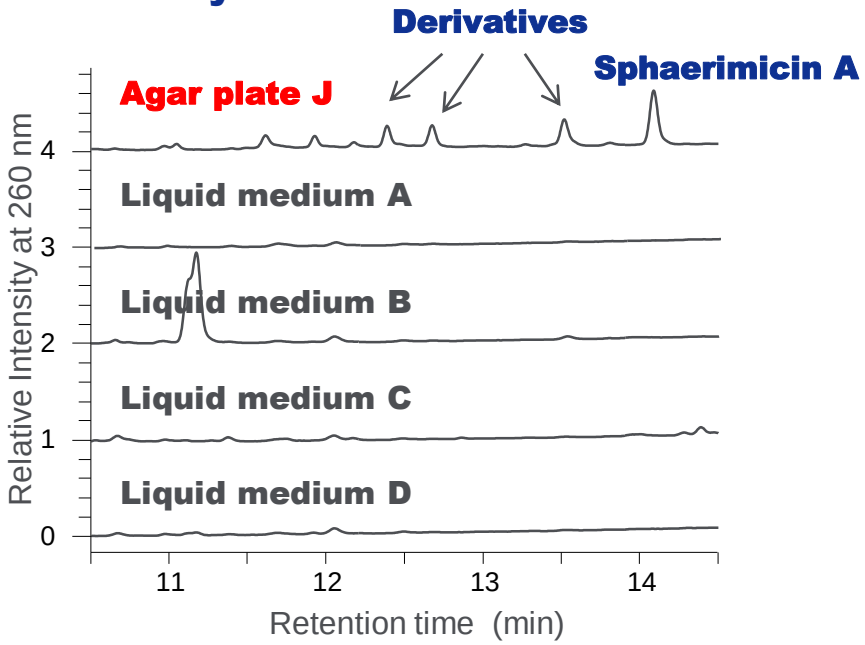
There were several ORFs (pink) of which homologs are involved in other translocase I inhibitor biosynthesis

Highly homologous to known SHMTs!

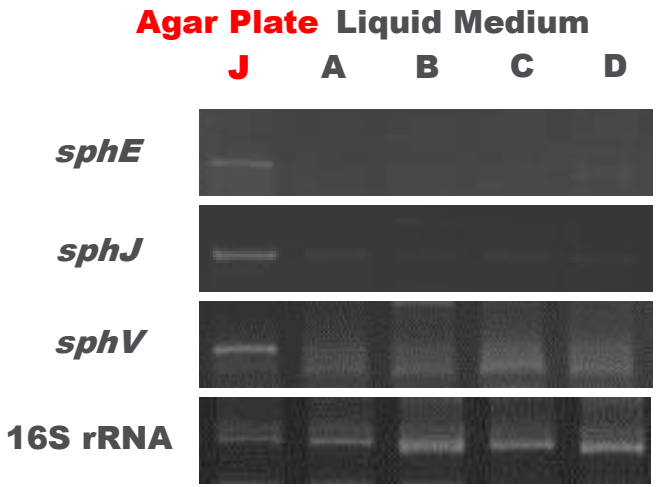
Results

V. Cultivation and Activation

HPLC Analysis



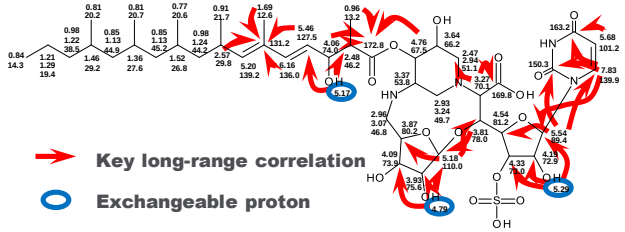
RT-PCR Analysis



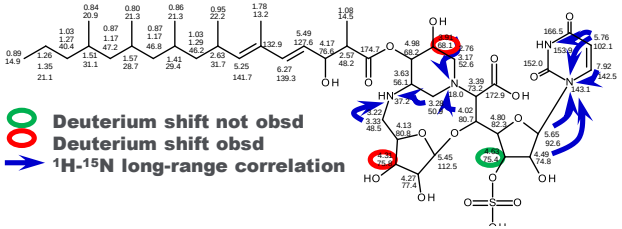
Only when *Sphaerisporangium* sp. SANK 60911 was cultivated on agar plate, products with the characteristic absorption at 260 nm and the expression of some biosynthetic genes were detected

Results

VI. Structural Elucidation



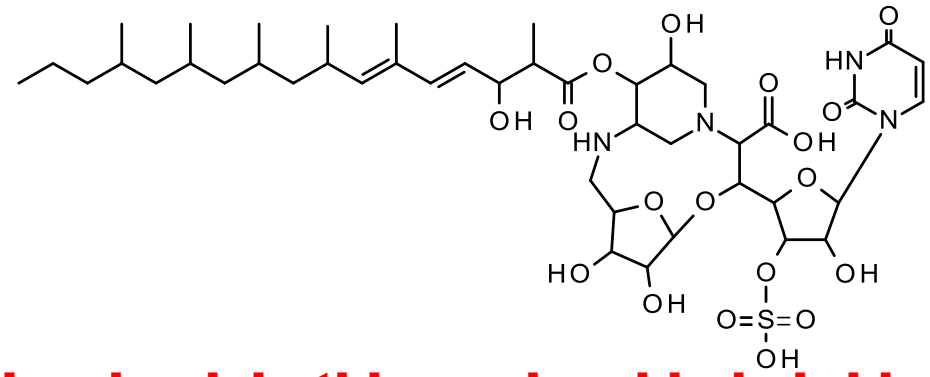
Sphaerimicin A in DMSO-d_6



Sphaerimicin A in CD_3OD

***O*-sulfate group substituted at C-3'**

Planar structure of sphaerimicin A



Novel polyketide-nucleoside hybrid antibiotic with objective uridine moiety and unique piperidine ring system

ご清聴有難うございました